INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/08847

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GIBSON U E M ET AL: "A NOVEL METHOD FOR REAL TIME QUANTITATIVE RT-PCR" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 995-1001, XP000642796 ISSN: 1088-9051 the whole document	1-9,14, 15
A	WOUDENBERG T M ET AL: "QUANTITATIVE PCR BY REAL TIME DETECTION" PROCEEDINGS OF THE SPIE, vol. 2680, 1 January 1996 (1996-01-01), XP000197422 the whole document	1-9,14, 15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2000	04/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 623 682 A (BECTON DICKINSON CO) 9 November 1994 (1994-11-09) page 5, line 24 - line 34	1-9,14, 15
A	WO 95 34684 A (UNIV GEORGETOWN) 21 December 1995 (1995-12-21) claims 1-4	1,9,13
A	SECCHIERO P ET AL: "QUANTITATIVE PCR FOR HUMAN HERPESVIRUSES 6 AND 7" JOURNAL OF CLINICAL MICROBIOLOGY,US,WASHINGTON, DC, vol. 33, no. 8, 1 August 1995 (1995-08-01), pages 2124-2130, XP000564243 ISSN: 0095-1137 the whole document	9-11
A	KENNEDY MM ET AL: "Identification of HHV8 in early Kaposi's sarcoma: IMPLICATIONS FOR kAPOSI'S SARCOMA PATHOGENESIS" MOLECULAR PATHOLOGY, vol. 51, no. 1, February 1998 (1998-02), pages 14-20, XP000892767 the whole document	1,9,12
A	ZIMMERMANN K ET AL: "TECHNICAL ASPECTS OF QUANTITATIVE COMPETITIVE PCR" BIOTECHNIQUES,US,EATON PUBLISHING, NATICK, vol. 21, no. 2, 1 August 1996 (1996-08-01), pages 268-270,272,27, XP000597689 ISSN: 0736-6205	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/08847

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0623682 A	09-11-1994	US 5457027 A	10-10-1995
		AU 675503 B	06-02-1997
		AU 6058094 A	10-11-1994
		CA 2121658 A,C	06-11-1994
		DE 69420454 D	14-10-1999
		DE 69420454 T	23-12-1999
		JP 6343497 A	20-12-1994
		SG 50707 A	20-07-1998
		US 5470723 A	28-11-1995
		US 5561044 A	01-10-1996
		US 5736365 A	07-04-1998
		US 5840487 A	24-11-1998
WO 9534684 A	21-12-1995	AU 692792 B	18-06-1998
		AU 7512394 A	05-01-1996
		JP 10505224 T	26-05-1998

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 June 2000 (23.06.00)	
International application No. PCT/EP99/08847	**Applicant's or agent's file reference** SCB 519 PCT
International filing date (day/month/year) 17 November 1999 (17.11.99)	**Priority date** (day/month/year) 17 November 1998 (17.11.98)
Applicant LOCATELLI, Giuseppe et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

24 May 2000 (24.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. Villet
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB 519 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08847	17/11/1999	17/11/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant FONDAZIONE CENTRO SAN RAFFAELE DEL MONTE TABOR		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/05/2000	16.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hinchliffe, P Telephone No. +49 89 2399 8431

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08847

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-19	as originally filed				

Claims, No.:

1-18	as received on	27/11/2000	with letter of	24/11/2000	

Drawings, sheets:

1/6-6/6	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Items V and VIII

1. The claims concern methods, a use and a kit designed to calibrate a PCR reaction using an internal control added to the sample to be PCRed (the calibrator). The calibrator molecule is defined as having the same nucleotide composition as a region of the target sequence but in a randomised order and having a similar Tm. The closest prior art is considered to be the document cited un the ISR by Gibson et al. It differs from the present method in that the internal control sequence differs by having a totally random sequence but maintaining the G-C content and Tm. Novelty under Art 33(2) PCT is therefore acknowledged as the internal control(calibrators used in the methods are different.

2. Inventive step is not acknowledged. It is alleged that the present method of performing an assay with quantitation in one tube is not shown in D1 because D1 suggests that a calibration curve is necessary where a wide dynamic range of sample inputs is concerned. However it is considered that D1 is pertinent as no proof is provided that the present method would provide quantitation under the same situations. Furthermore D1 notes on page 995 that as a general rule the internal control should use the same primers and contain a similar G-C content and be of the same or similar length. It is known that G-C content affects the Tm (see for example Genes II by Lewin or any standard text on molecular biology). Consequently the proviso that a similar Tm be maintained in the calibrator used in the claimed method is effectively the same as what is given in D1, i.e. that the G-C content is critical for accurate quantitiation. Consequently the method is not inventive contrary to Article 33(3) PCT because the calibrator molecule design is not surprising.

3. Contrary to Article 6 PCT, the term "similar Tm", found in claim 1, is not clear. Furthermore both claims 17 and 18 are not clear because the calibrator molecules are not defined.

CLAIMS

1) A method for the quantitative detection of a nucleic acid (target) from a sample, which comprises the following steps:

a) extraction of the nucleic acid from the sample with another nucleic acid (calibrator) previously added to the sample itself, said calibrator having the same sequence of the target nucleic acid, with the exception of one or more regions which in the target nucleic acid hybridize with a probe labeled with a reporter and a quencher, or which hybridize with said probe and in addition with two or more primers, such regions having each other different, randomized nucleotide sequences and a similar Tm, and

b) mixing the extracted target nucleic acid and calibrator with primers (forward and reverse) annealing to the corresponding regions on the calibrator and on the target nucleic acid or in addition with primers annealing to the randomized regions on the calibrator, as specified in (a), with probes annealing to the target nucleic acid and to the corresponding randomized region on the calibrator, said probes bearing a reporter and a quencher, and with a nucleic acid polymerase with 5'-3' nuclease activity, in suitable conditions to carry out a polymerization reaction, and

c) determination of the signal associated with the reporters released due to the 5' polymerase nuclease activity.

2) Method according to claim 1, wherein the calibrator Tm is comprised in the ±4°C range of the target nucleic acid Tm.

3) Method according to claims 1-2, wherein the 5' end of the probes is 1 to 30 nucleotides from the 3' end of the forward primer.

4) Method according to claims 1-3, wherein the probes have the 3' end blocked in order to prevent the extension by the polymerase.

5) Method according to claims 1-4, wherein said nucleic acids, said probes and said primers are DNA sequences, and the nucleic acid polymerase is thermostable DNA polymerase with 5'-3' nuclease activity.

6) Method according to claims 1-5, wherein the probes have a Tm higher than that of the primers.

7) Method according to claim 6, wherein said probes include 18 to 30 nucleotides.

8) Method according to claims 1-7, wherein said probes include a quencher label able to reduce or to avoid the reporter label fluorescence when the probes are free in solution.

9) Method according to any of the preceding claims, wherein the target nucleic acid is genomic nucleic acid of the viruses HHV-6, HHV-7, HHV-8 and HIV.

10) Method according to claim 9, wherein the virus is HHV-6, the forward primer has the sequence 5' CAAAGCCAAATTATCCAGAGCG 3', the reverse primer the sequence 5' CGCTAGGTTGAGGATGATCGA 3', the target nucleic acid probe the sequence 5' CACCAGACGTCACACCCGAAGGAAT 3', and the calibrator probe the sequence 5' TACGCAACGCCAACAGACCTAGCGA 3'.

11) Method according to claim 9, wherein the virus is HHV-7, the forward primer has the sequence 5' AGCGGTACCTGTAAAATCATCCA 3', the reverse primer the sequence 5' AACAGAAACGCCACCTCGAT 3', the target nucleic acid probe the sequence 5' ACCAGTGAGAACATCGCTCTAACTGGATCA 3', and the calibrator probe the sequence 5' TAAGCCCTGACCGCACGGGTATAATACTAA

3'.

12) Method according to claim 9, wherein the virus is HHV-8, the forward primer has the sequence 5' GTCCAGACGATATGTGCGC 3', the reverse primer the sequence 5' ACTCCAAAATATCGGCCGG 3', the target nucleic acid probe the sequence 5' CATTGGTGGTATATAGATCAAGTTCCGCCA 3', and the calibrator probe the sequence 5' ACTATTCCATGCGGAATTCGAGCATAGTTG 3'.

13) Method according to claim 9, wherein the virus is HIV-1, the forward primer has the sequence 5' TACTGACGCTCTCGCACC 3', the reverse primer the sequence 5' TCTCGACGCAGGACTCG 3', the target nucleic acid probe the sequence 5' ATCTCTCTCCTTCTAGCCTCCGCTAGTCAA 3', and the calibrator probe the sequence 5'ACTCTCAGCGGCATTCTCCTCACTTCTACT 3'.

14) Use of a calibrator, as defined in the preceding claims, in a method for the quantitative detection of a nucleic acid sample.

15) Kit for the quantitation of a nucleic acid from a sample, comprising one or more calibrators, a probe specific for each target nucleic acid and a probe specific for the calibrator, two or more primers and a thermostable nucleic acid polymerase with 5'-3' nuclease activity.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68, 1/70

A1

(11) International Publication Number: **WO 00/29613**

(43) International Publication Date: 25 May 2000 (25.05.00)

(21) International Application Number: PCT/EP99/08847

(22) International Filing Date: 17 November 1999 (17.11.99)

(30) Priority Data:
MI98A002491 17 November 1998 (17.11.98) IT

(71) Applicant *(for all designated States except US)*: FONDAZIONE CENTRO SAN RAFFAELE DEL MONTE TABOR [IT/IT]; Via Olgettina, 60, I–20132 Milano (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LOCATELLI, Giuseppe [IT/IT]; Via Olgettina, 58, I–20132 Milano (IT). LUSSO, Paolo [IT/IT]; Via Olgettina, 58, I–20132 Milano (IT). MALNATI, Mauro [IT/IT]; Via Olgettina, 58, I–20132 Milano (IT). SALVATORI, Francesca [IT/IT]; Via Olgettina, 58, I–20132 Milano (IT). SCARLATTI, Gabriella [IT/IT]; Via Olgettina, 58, I–20132 Milano (IT).

(74) Agent: MINOJA, Fabrizio; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I–20122 Milano (IT).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR THE QUANTITATIVE DETECTION OF NUCLEIC ACIDS

(57) Abstract

Provided herein is a method for the quantitative detection of nucleic acids based on the use of a calibrator, suitable primers and probes, and a nucleic acid polymerase with 5'–3' nuclease activity.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/EP 99/08847

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WOUDENBERG T M ET AL: "QUANTITATIVE PCR BY REAL TIME DETECTION" PROCEEDINGS OF THE SPIE, vol. 2680, 1 January 1996 (1996-01-01), XP000197422 the whole document	1-9,14, 15

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2000	04/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 95 34684 A (UNIV GEORGETOWN) 21 December 1995 (1995-12-21) claims 1-4	1,9,13
A	SECCHIERO P ET AL: "QUANTITATIVE PCR FOR HUMAN HERPESVIRUSES 6 AND 7" JOURNAL OF CLINICAL MICROBIOLOGY,US,WASHINGTON, DC, vol. 33, no. 8, 1 August 1995 (1995-08-01), pages 2124-2130, XP000564243 ISSN: 0095-1137 the whole document	9-11
A	KENNEDY MM ET AL: "Identification of HHV8 in early Kaposi's sarcoma: IMPLICATIONS FOR kAPOSI'S SARCOMA PATHOGENESIS" MOLECULAR PATHOLOGY, vol. 51, no. 1, February 1998 (1998-02), pages 14-20, XP000892767 the whole document	1,9,12
A	ZIMMERMANN K ET AL: "TECHNICAL ASPECTS OF QUANTITATIVE COMPETITIVE PCR" BIOTECHNIQUES,US,EATON PUBLISHING, NATICK, vol. 21, no. 2, 1 August 1996 (1996-08-01), pages 268-270,272,27, XP000597689 ISSN: 0736-6205	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ional Application No
PCT/EP 99/08847

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0623682 A	09-11-1994	US 5457027 A	10-10-1995
		AU 675503 B	06-02-1997
		AU 6058094 A	10-11-1994
		CA 2121658 A,C	06-11-1994
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		DE 69420454 T	23-12-1999
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		SG 50707 A	20-07-1998
		US 5470723 A	28-11-1995
		US 5561044 A	01-10-1996
		US 5736365 A	07-04-1998
		US 5840487 A	24-11-1998
WO 9534684 A	21-12-1995	AU 692792 B	18-06-1998
		AU 7512394 A	05-01-1996
		JP 10505224 T	26-05-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SCB 519 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 08847	17/11/1999	17/11/1998

Applicant

FONDAZIONE CENTRO SAN RAFFAELE DEL MONTE TABOR

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/08847

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GIBSON U E M ET AL: "A NOVEL METHOD FOR REAL TIME QUANTITATIVE RT-PCR" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 995-1001, XP000642796 ISSN: 1088-9051 the whole document ---	1-9,14, 15
A	WOUDENBERG T M ET AL: "QUANTITATIVE PCR BY REAL TIME DETECTION" PROCEEDINGS OF THE SPIE, vol. 2680, 1 January 1996 (1996-01-01), XP000197422 the whole document ---	1-9,14, 15
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2000	04/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 623 682 A (BECTON DICKINSON CO) 9 November 1994 (1994-11-09) page 5, line 24 - line 34 ---	1-9,14, 15
A	WO 95 34684 A (UNIV GEORGETOWN) 21 December 1995 (1995-12-21) claims 1-4 ---	1,9,13
A	SECCHIERO P ET AL: "QUANTITATIVE PCR FOR HUMAN HERPESVIRUSES 6 AND 7" JOURNAL OF CLINICAL MICROBIOLOGY,US,WASHINGTON, DC, vol. 33, no. 8, 1 August 1995 (1995-08-01), pages 2124-2130, XP000564243 ISSN: 0095-1137 the whole document ---	9-11
A	KENNEDY MM ET AL: "Identification of HHV8 in early Kaposi's sarcoma: IMPLICATIONS FOR kAPOSI'S SARCOMA PATHOGENESIS" MOLECULAR PATHOLOGY, vol. 51, no. 1, February 1998 (1998-02), pages 14-20, XP000892767 the whole document ---	1,9,12
A	ZIMMERMANN K ET AL: "TECHNICAL ASPECTS OF QUANTITATIVE COMPETITIVE PCR" BIOTECHNIQUES,US,EATON PUBLISHING, NATICK, vol. 21, no. 2, 1 August 1996 (1996-08-01), pages 268-270,272,27, XP000597689 ISSN: 0736-6205 -----	